# Measurement of Iron in Egg Yolk: An Instrumental Analysis Experiment Using Biochemical Principles

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The American Chemical Society's Committee for Professional Training (CPT) initiated steps around 1996 to implement the requirement of exposure to biochemistry in all approved chemistry programs. Since then, several experiments have been published in this *Journal (1–9)* that use biochemical aspects in the analytical chemistry track. While some of these experiments analyze food and water, none of them use eggs or egg yolks as the sample. A literature search reveals three experiments in this *Journal (10–12)* that use eggs in the biochemistry and organic laboratories for the extraction of phosphatidyl choline and egg white lysozyme.

In the accepted method for the determination of iron in eggs, the shelled, raw egg is first dry ashed or acid-digested to destroy the organic portion, and the residual mineral portion is dissolved in acid and analyzed by flame atomic absorption spectrometry or inductively coupled plasma atomic emission spectrometry *(13)*. We have developed an experiment to determine the iron content in egg yolk using chemical extraction of the iron and visible spectrophotometry of the 1,10-phenanthroline complex for quantification. Spectrophotometric studies of the Fe(II)–1,10-phenanthroline complex have been used in experiments such as the assay of iron in vitamin tablets *(14)* and the slope–ratio method for the determination of the formula of the complex *(15)*. We use only the yolk of an egg because it contains almost all of the iron in an egg and the extraction is more efficient than with the whole egg. The experiment is suitable for the instrumental analysis portion of the analytical chemistry course and serves to demonstrate to the students the chemical and biochemical principles that can be used for the extraction of a mineral from a complex organic matrix.

Chymotrypsin is an efficient enzyme that uses covalent catalysis to cleave peptide bonds *(16)* and, in our method, we use a solution of α-chymotrypsin to release the $Fe^{3+}$ bound to phosphovitin in the yolk. Next, the $Fe^{3+}$ is reduced to $Fe^{2+}$ and allowed to form the characteristic orange–red complex with 1,10-phenanthroline. Finally, the proteins are precipitated and centrifuged at low temperature, and the iron–phenanthroline complex in the supernatant is quantified by UV–vis absorption spectrophotometry.

## Procedure

The egg yolk is separated from the albumin, weighed, and stirred well with a magnetic stir bar. Chymotrypsin solution and hydroxylamine hydrochloride are added to a weighed aliquot of the yolk. We have found that, owing to the complex biological matrix of the egg yolk, a large volume of 1,10-phenanthroline solution has to be added for complete complex formation and that the addition of solid 1,10-phenanthroline works better. The addition of ethanol is also required in this experiment to stabilize the complex *(17)*. An aliquot of the stirred egg yolk is subjected to the same procedure as above but without the addition of 1,10-phenanthroline and serves as the blank in the spectrometric measurement.

After color development is complete, trichloroacetic acid solution is added, which immediately precipitates the proteins. The solution is centrifuged in a refrigerated centrifuge. The supernatant is transferred to a volumetric flask and made up to the mark.

We find it significant that although complex formation between $Fe^{2+}$ and 1,10-phenanthroline is pH dependent, the intensity of the color is not affected by the addition of trichloroacetic acid to precipitate the proteins. Trichloroacetic acid reduces the pH of the sample to about 2, but the measured absorbance value of a sample at the wavelength of maximum absorption does not change for several hours. The complex has a high formation constant of the order of $1 \times 10^{21}$ *(18)*, and has been found to be stable in the pH range 1.5–9.0 *(19)*. We conclude that the thermodynamic stability prevails over the kinetic dissociation of the complex during the time period of the experiment. We do find that the intensity of the color fades and the measured absorbance value decreases after the sample is allowed to stand overnight.

The absorption spectrum of each standard, the blank, and each sample solution is recorded using a UV–vis scanning spectrophotometer, and a calibration curve is constructed using absorbance at the wavelength of maximum absorption versus concentration of the standards in mol/L. The concentration of Fe(II) in the sample solution is calculated using the equation of the straight line, and the iron content of the egg yolk is determined. Details of the student procedure and instructor notes including directions for the preparation of solutions are given in the online supplement.

## Hazards

The Material Safety Data Sheets on the chemicals used in this experiment emphasize caution with respect to ingestion, inhalation, and skin and eye contact (1,10-phenanthroline, hydroxylamine hydrochloride, trichloroacetic acid, chymotrypsin, ethanol, ferrous ammonium sulfate, glacial acetic acid, sodium acetate trihydrate, sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dodecahydrate). Preparation of solutions must be done in the chemical hood while wearing gloves. Moreover, 1,10-phenanthroline and trichloroacetic acid are known to be toxic in the environment. Disposal should be done in accordance with environmental guidelines.

## Results and Conclusion

An Internet search reveals a range of values from 0.4 to 0.7 mg for the iron content of an egg yolk. The USDA National Nutrient Database Web site *(20)* indicates that the iron content of a raw, fresh, egg yolk is 0.45 mg. We have carried out this experi-

ment for the past three years and our typical results have varied between 0.3 and 0.5 mg. Obviously, the experiment cannot be graded on accuracy because there is no "true" answer. The value of this experiment lies in the use of a sample that is of interest to students because of their familiarity with its nutritional importance and in illustrating to students the use of chemical and biochemical principles in the extraction of an inorganic mineral from a complex biological matrix.

## Acknowledgments

The authors are very grateful to Harry L. Price for invaluable suggestions during the planning of this experiment.

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